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<u>L14</u>	ligand or polylysine	102343	<u>L14</u>
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<u>L11</u>	l5 with l1	480	<u>L11</u>
<u>L10</u>	l7 and l6	17	<u>L10</u>
<u>L9</u>	L7 same l6	0	<u>L9</u>
<u>L8</u>	L7 with l6	0	<u>L8</u>
<u>L7</u>	gene transfer or gene therapy or gene delivery	35887	<u>L7</u>
<u>L6</u>	L5 with l4	321	<u>L6</u>
<u>L5</u>	polynucleotide or dna or nucleic or plasmid	203333	<u>L5</u>
<u>L4</u>	L3 with l2	6245	<u>L4</u>
<u>L3</u>	metal ion	87436	<u>L3</u>
<u>L2</u>	Chelating or chelator	56080	<u>L2</u>
<u>L1</u>	PNA with (linker or spacer)	706	<u>L1</u>

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File: USPT

Jan 30, 2001

DOCUMENT-IDENTIFIER: US 6180767 B1

TITLE: Peptide nucleic acid conjugates

Detailed Description Text (30):

While the ligand molecule may be attached directly to the PNA oligomer, it is preferred for steric reasons that the two molecules are coupled in a spaced relation, through inclusion of a linker moiety. Preferably, the ligand is separated from the DNA oligomer by a distance of from about 10 to about 30 .ANG.. Linker moieties are selected accordingly. The linker may comprise any chemical group which is compatible with the ligand and PNA oligomer and which does not adversely affect either conjugate uptake or oligomer hybridization to the target nucleic acid segment.

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L15: Entry 7 of 13

File: USPT

Nov 6, 2001

US-PAT-NO: 6312956

DOCUMENT-IDENTIFIER: US 6312956 B1

TITLE: Nuclear targeted peptide nucleic acid oligomer

DATE-ISSUED: November 6, 2001

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Lane; Kirk B.	Brentwood	TN		

US-CL-CURRENT: 435/455; 514/44, 536/23.1, 536/24.5

CLAIMS:

What is claimed is:

1. A composition, comprising:

(a) a nuclear localization sequence;

(b) a peptide nucleic acid oligomer; and

(c) a membrane transport sequence, wherein the nuclear location sequence, the peptide nucleic acid oligomer, and the membrane transport sequence are combined in any order.

2. The composition of claim 1, wherein the nuclear localization sequence and the peptide nucleic acid oligomer are linked by a single peptide bond.

3. The composition of claim 2, further comprising a nucleic acid including a complementary region, wherein at least a portion of the peptide nucleic acid oligomer is annealed to the complementary region of the nucleic acid.

4. The composition of claim 1, further comprising a nucleic acid including a complementary region, wherein at least a portion of the peptide nucleic acid oligomer is annealed to the complementary region of the nucleic acid.

5. A composition comprising a nuclear localization sequence linked to a peptide nucleic acid by a single peptide bond.

6. A composition comprising:

(a) a nuclear localization sequence;

(b) a peptide nucleic acid oligomer; and

(c) a double-stranded vector including a coding strand, a non-coding strand having a binding site, and a promoter operably linked to a coding sequence; wherein at least a portion of the peptide nucleic acid oligomer is complementary to the binding site and annealed to the binding site in an area

of the promoter.

7. The composition of claim 6, wherein the nuclear localization sequence and the peptide nucleic acid are combined by a single peptide bond.

8. The composition of claim 7, wherein the composition further comprises a membrane transport sequence.

9. A method of manufacturing a composition, comprising:

(a) providing amino acids and peptide nucleic acids; and

(b) forming peptide bonds between the amino acids and the peptide nucleic acids in a sequence specific order to form a PNA amino acid chimera including at least one peptide nucleic acid oligomer segment and at least one peptide segment having a nuclear localization sequence.

10. The method of claim 9, wherein the PNA amino acid chimera further includes a membrane transport sequence.

11. The method of claim 10, wherein the forming step comprises solid phase synthesis.

12. The method of claim 10, further comprising annealing the PNA amino acid chimera to a nucleic acid wherein the PNA amino acid chimera includes a sequence of peptide nucleic acids that are complementary to a region of the nucleic acid.

13. The method of claim 12, wherein the nucleic acid comprises an expression vector including a promoter operably linked to a coding sequence, a coding strand and a non-coding strand; and wherein the region of the nucleic acid is on the non-coding strand of the vector in an area of the promoter.

14. The method of claim 13, further comprising:

(a) contacting a eukaryotic cell with the composition of claim 13; and

(b) expressing an expression product from the vector in the eukaryotic cell.

15. The method of claim 14, wherein the eukaryotic cell is non-human.

16. The method of claim 14, wherein the eukaryotic cell is quiescent.

17. The method of claim 14, further comprising collecting the expression product from the eukaryotic cell.

18. The method of claim 9, further comprising annealing the PNA amino acid chimera to a nucleic acid wherein the PNA amino acid chimera includes a sequence of peptide nucleic acids that are complementary to a region of the nucleic acid.

19. The method of claim 9, further comprising:

(a) annealing the PNA amino acid chimera to a yeast expression vector including a coding sequence encoding an expression product and a peptide nucleic acid binding site that is complementary to a sequence of peptide nucleic acids of the PNA amino acid chimera;

(b) transfecting a yeast cell with the PNA amino acid chimera annealed to the yeast expression vector;

(c) expressing the expression product in the yeast cell; and

(d) collecting the expression product.

20. A method of manufacturing a composition, comprising

(a) providing amino acids and peptide nucleic acids; and

(b) combining the amino acids and the peptide nucleic acids in a sequence specific order to form a PNA amino acid chimera including at least one peptide nucleic acid oligomer, a nuclear localization sequence, and a membrane transport sequence.

21. The method of claim 20, further comprising annealing the PNA amino acid oligomer to a complementary region of a nucleic acid.

22. The method of claim 21, wherein the nucleic acid comprises an expression vector including a promoter operably linked to a coding sequence, a coding strand and a non-coding strand; and wherein the complementary region of the nucleic acid is on the non-coding strand of the vector in an area of the promoter.

23. The method of claim 22, further comprising:

(a) contacting a eukaryotic cell with the composition of claim 22; and

(b) expressing an expression product from the vector in the eukaryotic cell.

24. The method of claim 23, wherein the eukaryotic cell is non-human.

25. The method of claim 23, wherein the eukaryotic cell is quiescent.

26. The method of claim 23, further comprising collecting the expression product from the eukaryotic cell.

27. A method for inhibiting an expression of an expression product from an endogenous nucleic acid in a nucleus of a eukaryotic cell, comprising:

(a) transfecting the eukaryotic cell with a composition including a PNA amino acid chimera having a nuclear localization sequence and a segment of peptide nucleic acid residues that are complementary to a binding site on a coding strand of the endogenous nucleic acid in an area of the nucleic acid encoding the expression product; and

(b) allowing the segment of peptide nucleic acid residues of the composition to anneal to the binding site on the coding strand, thereby inhibiting the expression of the expression product.

28. The method of claim 27, wherein the coding strand of the endogenous nucleic acid includes a mutation.

29. The method of claim 27, wherein the nucleic acid is of viral origin.

30. The method of claim 27, wherein the PNA amino acid chimera further includes a membrane transport sequence.

31. The method of claim 27, wherein the nuclear localization sequence is linked to the peptide nucleic acid oligomer by a single peptide bond.

32. The method of claim 31, wherein the coding strand of the endogenous nucleic acid includes a mutation.

33. The method of claim 31, wherein the nucleic acid is of viral origin.
34. The method of claim 31, wherein the PNA amino acid chimera further includes a membrane transport sequence.
35. The method of claim 34, wherein the expression product is a tumor promoter.
36. A method for increasing a production of an expression product from an endogenous nucleic acid in a nucleus of a eukaryotic cell, comprising:
- (a) transfecting the eukaryotic cell with a composition including a PNA amino acid chimera having a nuclear localization sequence and a sequence of peptide nucleic acid residues that are complementary to a binding site on a non-coding strand of the endogenous nucleic acid in an area encoding the expression product; and
 - (b) allowing the sequence of peptide nucleic acid residues of the composition to anneal to the binding site on the non-coding strand, thereby increasing the production of the expression product.
37. The method of claim 36, wherein the nucleic acid is of viral origin.
38. The method of claim 36, wherein the PNA amino acid chimera further includes a membrane transport sequence.
39. The method of claim 36, wherein the nuclear localization sequence is linked to the peptide nucleic acid oligomer by a single peptide bond.
40. The method of claim 36, further comprising collecting the expression product from the eukaryotic cell.
41. The method of claim 40, wherein the PNA amino acid chimera further includes a membrane transport sequence.
42. The method of claim 36, wherein the expression product is a tumor suppresser.
43. The method of claim 42, wherein the PNA amino acid chimera further includes a membrane transport sequence.
44. A kit comprising packaged together:
- (a) a composition including a nuclear localization sequence linked to a peptide nucleic acid oligomer by a peptide bond, wherein the peptide nucleic acid oligomer includes a binding segment;
 - (b) a double-stranded plasmid including a transcriptional promoter operably linked to a multiple cloning site and a binding site that is complementary to the binding segment, wherein the binding site is on a non-coding strand of the plasmid in an area of the promoter; and
 - (c) and a set of instructions for annealing the composition to the plasmid to form a nuclear targeted transfection complex.
45. The kit of claim 44, wherein the instructions further describe transfecting a eukaryotic cell with the nuclear targeted transfection complex.
46. The kit of claim 44 wherein the composition further includes a membrane transport sequence linked to the nuclear localization sequence, the peptide nucleic acid oligomer, or both the nuclear localization sequence and the peptide nucleic acid oligomer.